have learnt that MMP inhibitors could produce side effects and have not yet demonstrated a major reduction in the progression of the disease. Certain MMPs, such as MMP-13 and ADAMTS proteases, such as ADAMTS5, have been selected as being the most attractive targets for the treatment of OA. The main reason(s) for choosing selective inhibition, instead of a broad inhibition, is based on the hypothesis that by doing so a certain number of side effects that could potentially be related to a broad MMP inhibition can be avoided.

Another possible way to inhibit the effect of certain catabolic factors lies in the inhibition of certain intracellular signaling pathways such as the protein kinase cascades. The kinases, which constitute the most appealing targets, include p38, ERK 1/2, and SAPK/JNK. A recent study showed that an orally active ERK 1/2 selective inhibitors could very significantly and effectively reduce the progression of lesions in the rabbit OA model by inhibiting the synthesis of catabolic factors, such as MMPs. Another emerging field of research focuses on the development of molecules that can inhibit the binding of the transcription factors at the DNA level. The major transcription factors targeted for the inhibition of cytokines and MMP synthesis are NF-κB and AP-1.

On the subject of cell signaling, another interesting target are the ligands to a group of nuclear transcription factors, the peroxisome proliferator-activated receptor gamma (PPARγ), which act as anti-inflammatory agents. In vivo, synthetic PPARγ ligands were found to protect against structural damages in the collagen-induced arthritis model and in OA models.

OA structural changes also involve modifications in the morphology of the surrounding bone. Subchondral bone remodeling is a well-recognized manifestation of OA. New data underline the concept that abnormal subchondral bone cell functions may contribute to the onset/progression of OA. Therapeutic effects of drugs that prevent the abnormal metabolism of subchondral osteoblasts on the progression of OA lesions is currently of major interest in the context of future DMOAD development.

Several new pharmacologic DMOAD agents are under investigation. Preclinical results are promising and demonstrate the possibility of retarding or inhibiting the progression of joint tissue structural changes. The tools to study such effects in the human population remain unsatisfactory. Existing radiologic methods are useful but this methodology is imperfect and time consuming. Faster and more accurate methods are needed to improve the investigation of new drugs that have the potential to modify the progression of OA.

In conclusion, several interesting new approaches for the treatment of this disease are now being explored. New classes of molecules that inhibit one or more of the disease processes of OA are under evaluation for their potential to alter the degenerative process

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BIOREACTORS FOR CARTILAGE TISSUE ENGINEERING

Ivan Martin

Introduction: Recent animal experiments have demonstrated that engineered cartilage tissues generated by culturing chondrocytes into 3D scaffolds provide functional templates for the orderly repair of critically sized osteochondral lesions. In order to reproducibly generate functional cartilage tissues starting from adult human cells, efforts have to be directed not only to the identification of stimulatory biochemical factors, but also to the development and use of controlled bioreactor systems, applying defined regimes of physical forces. In this work, we present some examples on the use of bioreactors for processes that are key for engineering of 3D cartilage tissues based on cells and scaffolds, namely the chondrocyte seeding into porous scaffolds, their efficient nutrition, and the pysical conditioning of the developing tissues.

Chondrocyte seeding and culture under perfusion: In the cell seeding process, cells must be utilized with maximum efficiency to minimize the biopsy size needed and/or the extent of cell expansion, and must be dispersed uniformly throughout the scaffold volume to form the basis for uniform tissue formation. To overcome limitations associated with the most commonly employed seeding techniques, we developed a bioreactor for the automated cell seeding of three-dimensional scaffolds by continuous perfusion of a cell suspension through the scaffold pores in oscillating directions. Perfusion seeding of chondrocytes into Polyactive foams (IsoTis OrthoBiologics, NL) or Hyaff$^{?}$-11 non-woven meshes (Fidia Advanced Biopolymers, IT) resulted in the highest fraction of viable cells within the foam pores, the greatest efficiency of seeding and the highest uniformity of cell distribution in comparison to the typically used static and spinner flask methods [1].

Constructs uniformly seeded by perfusion and then cultured statically for 2 weeks were highly heterogeneous in structure, consisting of a layer of cells and matrix at the periphery and an essentially void interior region. Instead, constructs cultured under prolonged perfusion were remarkably homogeneous, containing a uniform distribution of both cells and matrix throughout the cross-section [2].

Physical conditioning of cartilagel constructs: Application of dynamic compression to cell-polymer constructs could potentially improve the development of cartilaginous tissue *in vitro*. We exposed human articular chondrocytes-based cartilaginous constructs at different stages of maturation, as defined by the glycosaminoglycan (GAG) content, to intermittent compressive deformation for 3 days. Compression-induced changes in GAG synthesis and accumulation were positively correlated to the GAG content prior to loading, such that compression was stimulatory only for the most developed constructs. Therefore, under our experimental conditions, cyclic loading appears to be applicable for the enhancement of cartilaginous tissue development only in the late phases of tissue regeneration [3]. Our results also point out the possible use of bioreactors applying defined regimes of physical forces as a quality control tool for engineered cartilage, with the goal of defining when the tissues are sufficiently developed for immediate load bearing after implantation.

Conclusion: The reviewed studies indicate that bioreactors enable generation of cartilaginous tissue constructs and may contribute to understand the function of specific chemico-physical culture parameters on cartilage tissue development. In the future, bioreactors are expected to efficiently translate laboratory- to inductrial-scale cartilage tissue engineering, possibly providing an economically viable approach to the automated manufacture of functional cartilage grafts for broad clinical use [4].

References

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PAIN IN OSTEOARTHRITIS

Hans-Georg Schaible

Purpose: The most dominant subjective problem of the patient suffering from osteoarthritis is pain. In most cases pain is localized to the joint with OA but it can be referred to other joints. OA pain varies in intensity and is usually worsened by exercise

and relieved at rest. Some patients suffer from pain at night. OA pain is often episodic but may be constant in advanced OA. Often there is a poor relationship between pain and joint damage as assessed by X-ray, but some MRI studies revealed that painful osteoarthritic joints show more MRI-abnormalities such as synovial hypertrophy, synovial effusions and bone marrow edema lesions. Furthermore chronic inflammatory changes can be present. Finally, self-reported pain is often influenced by perceived helplessness, educational, social and psychological factors showing the complexity of chronic pain. To approach the pain problem it is necessary to study the principles of nociception in the joint, the processing of nociceptive sensory input from the joint in the central nervous system and the neuronal processes in the thalamocortical system that generate the pain response with its sensory and affective components.

Methods: In order to investigate pain mechanisms numerous methods are being used including morphological methods to visualize nerve fibres, electrophysiological methods to record from nociceptive neurons in the peripheral and central nervous system and approaches to assess pain behaviour. In humans brain imaging techniques are widely used to show the activity in the so-called thalamocortical pain matrix.

Results: Although osteoarthritis is a disease of cartilage it is unlikely that pain arises from nociceptive stimulation of cartilage itself because cartilage does not seem to be innervated. It is thought, therefore, that the pain is generated by nociceptive stimulation of nerve fibres in capsule, menisci, ligaments and subchondral bone. At these sites sensory endings of thin myelinated A delta- and unmyelinated C-fibres have been visualized. Electrophysiological recordings from nerve fibres in the joint nerve have revealed the response properties of A-delta and C-fibres to noxious stimuli. Nociceptive joint afferents are either polymodal responding to noxious mechanical stimuli and to mediators such as inflammatory mediators or they are silent not responding to mechanical stimulation under normal conditions. Importantly, nociceptive nerve fibres can be sensitized during pathological processes such as inflammation which results in enhanced responsiveness of polymodal nociceptors to mechanical stimulation (lowering of threshold, enhanced responses to suprathreshold stimuli) and in the induction of mechanosensitivity of silent nociceptors. Key to sensitization is the chemosensitivity of joint afferents. A-delta and C-fibres express receptors for numerous mediators that play an important role in processes of joint inflammation and osteoarthritis such as prostaglandins, bradykinin, cytokines and others. Inflammatory mediators activate second messenger systems in neurons that influence ion channels involved in stimulus transduction. Receptors in sensory nerve fibres can be upregulated under acute and chronic painful conditions. This process of peripheral sensitization can induce a state of central sensitization. An important amplification of the nociceptive processing through the process of central sensitization takes place in the spinal cord. Data from OA patients indicate that central sensitization is present in painful OA states. Ascending tracts convey the information from the spinal cord to the thalamocortical system where the conscious pain response is generated. Imaging data emphasize the existence of two systems that are involved in pain. The lateral thalamocortical system consisting of lateral thalamic relay nuclei and the cortical areas S1 and S2 produces the sensory discriminative pain response analysing the site, intensity and duration of painful stimuli. The medial thalamocortical system consisting of medial thalamic nuclei and cortical areas in the insula, the anterior cingulate gyrus and the prefrontal cortex produces the affective pain response which causes the suffering from pain.

Conclusions: OA pain under activated conditions such as acute episodes of inflammation or chronic inflammatory conditions is likely to result from peripheral and central sensitization. Whether additional mechanisms such as damage of nerve fibres and elements of neuropathic pain contribute to OA pain is being explored. Future progress in the understanding of OA pain is expected from techniques that allow to correlate pathological changes in the diverse joint structures with the experience of pain and from the work on experimental models that are relevant for human OA.

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NEW METHODS FOR MOLECULAR DISCOVERY IN OSTEOARTHRITIS: A FOCUS ON CELL SIGNALING

Richard F. Loeser Jr.

A better understanding of basic molecular mechanisms that regulate cartilage matrix destruction is needed in order to develop new treatments for osteoarthritis that have the potential to slow disease progression. It is well accepted that the chondrocyte is responsible for the progressive destruction and loss of its own matrix through the release of degradative enzymes accompanied by an inadequate repair response. This appears to result from an imbalance in chondrocyte anabolic and catabolic activity. Studies on the cell signaling pathways in chondrocytes that regulate anabolic and catabolic activity, including the effects of aging on these pathways, should define novel targets for correcting the imbalance. Genomics approaches have defined numerous genes that are up or downregulated in OA chondrocytes when compared to chondrocytes from normal joints. Expression of many of these genes may be regulated by a common set of key signaling intermediates that are activated in response to stimulation of chondrocytes by cytokines and by signals from the matrix mediated by the integrin family of receptors. Phosphorylation of specific tyrosine, serine and threonine residues mediated by kinases is a common mechanism for propagation of intracellular signals. Recently, the role of reactive oxygen species (ROS) as secondary messengers in cell signaling has become better understood. The reversible oxidation of specific cysteine residues to cysteine sulfenic acid acts to regulate the activity of many signaling proteins including kinases and phosphatases. In this workshop, cell signaling pathways relevant to the regulation of chondrocyte anabolic and catabolic activity will be reviewed with a focus on the methodologies used to study signal transduction. Advantages and pitfalls of various methodologies will be discussed with the goal of facilitating an interactive discussion on new methods that can be used to better understand basic molecular mechanisms regulating chondrocyte function.

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MRI IN OSTEOARTHRITIS, USEFUL FOR THE RESEARCHER AND CLINICIAN

Philip G. Conaghan

Magnetic resonance imaging (MRI) provides unparalleled ability to visualise all the structures involved in the whole-organ joint failure process that is osteoarthritis (OA). For the researcher it provides a new level of *in vivo* understanding of the key tissue pathology. The majority of MRI OA studies to date have focussed on the knee joint. Large cohort studies have demonstrated frequent abnormalities of cartilage, menisci, bone (bone marrow lesions, BML, and osteophytes), even when there is minimal radiographic abnormalities.

An important basic requirement for the researcher is valid methods of quantifying this joint pathology. Quantitative measures of cartilage and semi-quantitative score systems for all the knee structural features have been developed, and increasing data on their validity, reliability and sensitivity to change have been published over the last few years.

The next step is to understand the importance of these MRI